MICROSCOPY.[1]

Formol as a Preserving Fluid.[2]—If the four atoms of hydrogen in the simple organic combination, swamp gas or methan, be replaced by a hydroxyl group there may be formed, one after the other, partly by the separation of water, (1) methylalcohol, (2) methylenglycol, (3) formic acid, and (4) carbon dioxide. The process may be illustrated in the following manner:

$= CH_4 =$ methan.

$= CH_4O =$ methylalcohol.

$= CH_4O_2 =$ methylenglycol; and $CH_4O_2 - H_2O =$

$CH_2O =$ formaldehyde.

$= CH_2O_3$; and $CH_4O_3 - H_2O = CH_2O_2 =$ formic acid.

$= CH_4O_4$; and $CH_4O_4 - 2H_2O = CO_2 =$ carbon dioxide.

Of these five combinations it is formaldehyde that concerns us. It was discovered in 1863 by A. W. Hoffmann while passing wood spirit (methylalcohol) and air over a red hot platinum spiral. If the vapor

[1] Edited by C. O. Whitman, University of Chicago.

[2] The first half of this paper is a free translation of a paper by Prof. T. Blum in the Bericht. über d. senckenbergische naturf. Gesell. in Frankf. a. M., 1894, p. 195.—F. C. K.

is brought into water to its point of saturation, a 40 per cent solution of formaldehyde is obtained, which has long been known under the name of formol. The use of the termination "ol," here has been objected to as belonging especially to alcohols, but since we have to do not with the vapory formaldehyde of the discoverer, but with the hydrate, methylenglycol, an alcohol, this objection is not well founded. The first experiments as to the value of an aqueous solution of formaldehyde for the purposes of disinfection, hardening and preservation, were made with the solution under the name of formol; therefore, the general custom of priority giving the honor, I shall use the term formol.

Formol is a clear, slightly opalescent fluid with a sharp odor. By dilution of the fluid the odor is lessened and the liquid remains as clear as water. It is best kept in glass vessels. In metal ones it often becomes of dark brown color and must then be allowed to stand quietly in a glass vessel before diluting for use. From the quiet liquid there settles a light cloudy precipitate leaving the liquid clear. A change of formaldehyde to an insoluable paraformaldehyde, that has here and there been noted, I have never met with.

After my son, Dr. F. Blum, made the discovery that formaldehyde posessed besides its known antiseptic action, the noteworthy property of hardening animal tissues without their shrinking and without altering their microscopic structure or staining properties, formol appeared to me to be the preservative fluid for which I had long sought. Without loss of time, I began my experiments upon animal and plant objects. These gave within the short space of a few months such encouraging results that I did not hesitate to publish them in a preliminary paper.[3] Since then the experiments have been continued at the Museum der Senckenbergischen naturforchenden Gesellschaft, and in different places others have likewise tested the preservative properties of the fluid.

Among my experiments those that follow are the most important. To begin with, several human embryos were placed according to age in formol diluted with 10 and with 20 parts of water and were finely preserved. Even a fœtus of 8 months in which the placenta and egg membranes were left intact, had taken up so much formol that it was hardened in spite of the resistance of the chorion to the diffusion of the liquid. The amniotic fluid was darkened, but the surrounding liquid remained clear. Somewhat finer results were obtained with smaller embryos. In one about 14 cm. long with uninjured amnion, this being thinner,

[3] Zoologischer Anzeiger, 1893, No. 434.

the amniotic fluid did not become turbid. Through it each structural particular of the embryo and navel cord is easily recognized. The temporal artery shows through the transparent skin as a dark brown streak, while beneath it is seen the brain through its capsule. In an embryo a little larger (30 cm.), the fine hair and hair follicles are finely preserved. This last embryo was in a 1:20 solution.

Experiments with a corpse have not been made, yet the possibility of one keeping, may be with safety assumed. In order not to be obliged to inject the fluid, it might be necessary to employ the stronger, at least 1:10 solution.

Of the Mammalia, mouse, hamster and porpoise have been left in a 1:10 solution for over three-fourths of a year. The fluid has not been changed and yet remains perfectly clear, while the animals are well hardened, unaltered in form and color, and with the hair firmly in place. The mammalian eye as well as that of other vertebrates keeps better in formol than in alcohol. Still after a time a turbidness appears —more in the lens than in the cornea.

Reptilia and Amphibia preserve well. Frogs, in consequence of the entrance of the fluid into the subdermal space, appear swollen, but in other respects are unchanged.

For fishes, formol especially recommends itself. The mucous and slime remain clearly transparent, never forming the white, stringy mass arising in alcoholic preparations. Most fishes retain their colors more or less completely. Gold fish, to be sure, loose their color in very weak solutions, and the red spots of the trout become white with time. A solution diluted 1:10, 1:20, or 1:30, according to the size of the animals may be used. In a short time the animals are very nicely hardened.

From a number of invertebrates I may mention that snails, especially slugs, show their form and colors through the transparent slime. Insects, spiders and Crustacea preserve at least as well in formol as in alcohol.

Living Hirudinea are contracted more in formol than in alcohol; at least the contracted specimens are numerous and the extended ones few. The straw-yellow colors disappear sooner, while, on the other hand, orange-yellow, green, brown and black remain unchanged.

Two jelly fish (*Aurelia aurita*) killed in a 1:20 solution and kept one in a 1:30, the other in a 1:50 solution, were hardened without an alteration of form, color, or transparency. That kept in the 1:30 solution is the better, but neither have been long in the fluid.

Single organs or pieces of muscle are quickly hardened in formol. It is notable, as pointed out by my son,[4] that the coloring matter of the blood is distinctly retained. The blood courses, it is true, fade and finally to all appearances disappear, but if the preparation be placed in alcohol of not too great a strength (60–90 per cent.)—the stronger the quicker the reaction—the characteristic blood color returns and there is obtained an excellent representation of the branching of the vessels. The change from formol to alcohol and *vice versa* may be repeated always the same results.

Brain hardened in formol gives very fine results.[5] Pieces and even the entire brain are hardened very quickly and show the white and gray matter sharply differentiated from one another. Sections are said to be much better than those of chromic acid preparations.

As has been mentioned, neither the microscopic structure nor the staining properties of tissues are destroyed by formol. Almost all the organs and staining methods have been tried. In the preparations, cell body and cell structure, as well as the nucleus caught both in the resting state and in process of division are fixed, while the blood corpuscles are sharply marked off from their surroundings.

Hens' eggs have been tried and have in many ways led to very interesting results. An unbroken fresh egg in a 1:15 solution showed, after 8 days, the white forming about the yolk a mantle of an outer fluid and an inner slimy consistance. The yolk was hard, remaining fluid only in the middle. The hardening process here then is the reverse of that of cooking. On the day following, the yolk had become much harder, while the white was changed only after a long time and never neared the hardness of the yolk. Upon opening an egg after 38 days a faint odor of formol was perceived. The yolk was hard, sectionable, and showed an outer zone of 1½ mm. breadth and an inner beautiful yellow mass. The yolk was surrounded by a grayish, scarcely sectionable, gelatinous mantle in which the chalazea and germinal spot were plainly visible. About the mantle was a very slightly opalescent albuminous fluid.

A fresh egg with a small hole in it showed under like conditions the same phenomena, but within a shorter time, or about 17 days. After 68 days such an egg was noticeably harder. The firm white clung to

[4] Anatomischer Anzeiger, Vol. ix, No. 7.

[5] See Born, "Demonstration einer Anzahl in Formaldehyde (Formol) gehärteter menschlicher Gehirne." Mediz. Sektion d. schlesisch. Gesell. f. vaterl. Kultur., 1894.

the shell so that it shelled like a cooked egg. The white had the appearance of gelatin, was firm, and whitish-gray. The yolk was very hard and breakable.

Similar results to those with the unbroken eggs were obtained with uninjured eggs in formol vapor (a very few drops).

A cooked egg kept in formol vapor after 30 days appeared as fresh as though newly cooked, smelled of formol on the inside and had a sharp taste.

A fresh, unbroken egg that had been for 75 days in a 1:5 solution of formol was placed for 15 minutes in boiling water. Both yolk and white had the same appearance as in an uncooked egg that had been for a similar length of time in formol. In spite of the long cooking the white had not taken on that beautiful porcelain white appearance common to cooked eggs, and had not changed its firm gellatinous condition. Hence through the action of formol the white of an egg looses the property of coagulating by heat. If, as now assumed, egg-white bodies are those substances that are changed in chemical constitution by the action of formol, then the difference in the action upon the white and the yolk of the hen's egg offers a most worthy test for the study of different albuminous substances.

Experiments with plants were made in considerable number. In general the preservative action of formol upon the colors of flowers is less than the first experiments had led me to hope. Nevertheless, this means of preservation is a step in advance. Many flowers placed in formol during the summer were usable as demonstration preparations during the following winter. A passion flower in a 1:20 solution after nearly ten months, is still a beautiful preparation. Further, many composites, viz., such as had a yellow color, like *Helianthus argyrophyllum, Calendula officinalis,* etc., have been well preserved. Also a rhododendron flower (in 1:20), a rose (in 1:50), *Akebia quinata* (1:20), *Cornus mas* (1:20) and so on, have been changed in form and color but little. Fragant flowers and fruits turn the formol to an agreeably odiferous fluid. Chloropyll is not drawn out by the fluid, but the green color of tender leaves become pale with time. A *Dieffenbachia* with a bulb grown upon the spathe is almost faded, but forms never the less a fine preparation. Firm leaves like those of *Rhododendron* are altered but little. Fruits are well preserved. Blue grapes, currants, medlars, several species of *Cratægus, Cephalotaxus,* banana, different species of *Solanum, Magnolia tripetala,* strawberries, and *Mangifera indica,* that have been in formol ever since the fall of 1893, are nicely preserved. In a very few fruits the action of the preservative is injurious.

The use of a very dilute solution of formol works badly for the reason that from such a fluid the water is absorbed very decidedly. At least fruits became swollen more often than plants in the dilute solutions. Cherries, for instance, keep well in a 1:30 solution, but in one of 1:60 or 1:80 they burst open. The entrance of the fluid into the colored envelopes of flowers is also very noticeable. How dilute the solution may be for the different plants is difficult to say. It must be determined by experiment.

Of Cryptogamous plants I have till now experimented only with truffles (1:10) and young *Phallus impudicus* (1:30). This last was cut in two and forms an excellent preparation.

Cohn declares that formaldehyde forms an excellent means of preserving *Leuconostoc* and chromogenous bacteria since the jelly and color are not changed.[6]

The value of the fluid for preserving bacteria has been noticed by Hauser.[7] He shows that gelatin in which micro-organisms are grown is changed by formaldehyde vapor so that it will not become fluid, and that gelatin already peptonized becomes hard again in the vapor. Neither the gelatin nor the micro-organisms suffer a noticeable change, and the preparations can be kept for demonstration or museum purposes.

In microscopic sections of plants that have been in the preservative (1:20) for several months the cell wall, protoplasm and chlorophyll bodies appear as in fresh specimens.

I have not yet undertaken to determine the freezing point of the formol solution, but will remark that during the past cold winter in an unheated store room the diluted solution was not frozen, and that even in the open air at a temperature of—18° C the concentrated solution remained fluid.

In conclusion the properties of formol as a preservative medium may be summed up as follows:

Animal objects are hardened with shrinking, and without losing their microscopic structure or staining properties.

The natural form and color is preserved.

The eye remains much clearer than in alcohol.

The mucous of slime producing animals is not coagulated and remains transparent.

The coloring matter of blood in tissues apparently disappears, but may be quickly restored by a high per cent alcohol.

[6] Bot. Centralbl., Vol. lvii, No. 1, 1894.

[7] Münchener med. Wochenschrift, 1893, Nos, 30 & 35.

Plant structures are more or less well preserved; most fruits keep well.

Chlorophyll is not extracted, but after a long action of the fluid delicate leaves may be changed. The duration of the retention of other coloring matters is different with individual plants.

Microscopic sections of plants that have been a long time in formol give fine preparations.

Dilute formol is not combustible and is much cheaper than alcohol.

To the above experiments described by Blum may be added those of Dr. Th. Pintner, Dr. C. Krückmann, and a few notes of my own.

Dr. Pintner used a 1 per cent. solution of formaldehyde in sea water for Discomedusæ, *Æquorea* and *Aurelia* without their form being affected.[8] The same solution was used with sponges such as *Suberites dominicula* and *massa*, *Clathria coralloides*, *Aplysina aërophobia*, etc., with equally good results. But animals that contract much in killing must first be treated with Lo Bianco's Naples methods, and then transferred to the solution of formaldehyde. He found that all animals do not retain their color, as for instance, the red coloring matter of actinia and of *Comatula* is extracted.

Dr. Krückmann working with bacteria used stronger solutions and obtained the best results by combining corrosive sublimate with formalin.[9] To begin with, a formalin solution of moderate strength was used and this gradually increased until the specimens were in pure formalin. Bacterial cultures were fixed by placing them in an exciccator containing formalin instead of sulphuric acid, and in order to tan the surface of the medium, it was covered with a 1:10 solution of formalin containing 1 per cent of sublimate. This was later changed to a stronger solution of formalin and the tube hermetically sealed. By following this process he found that colors were much better preserved and the more or less inevitable crumpling very much diminished. The solution worked well with all media except potato.

The few experiments that have been performed by myself seem to indicate that too weak solutions of formalin have hitherto been used except in the bacterial experiments of Krückmann. The material used was what came nearest to hand, and consisted of a tree-frog, salamanders, earth worms, sow-bugs, myriapods, plant-lice, slugs, cat liver and blood, blood of salamander, nostoc and a pond scum. The solutions used varied from ¼ per cent. to pure formalin. Two species of

[8] Ver. zool.-bot. Ges. Wien, xliv, (1894), p. 8.

[9] Centralbl. f. Bakteriol. u. Parasiteuk., xv, (1894), pp. 851–7.

Plethodon placed in a 4 per cent. solution of formaldehyde (=10 per cent. of the commercial formalin) were soon killed, and on the day following the immersion were thoroughly hardened and after a week or so have not shrunken noticeably further than that the costal furrows are a little more strongly marked than in life. The reddish coloring of one of them is fully as fresh as when the animal was caught. A tree-frog placed in the same solution at the same time became somewhat swollen, but by cutting the skin in the abdominal region, the swelling was then shown to be due to an entrance of the fluid into the subdermal space as pointed out by Blum. The swollen tongue, which protruded from the mouth a little, would indicate, however, that there had been a swelling of some of the tissues. The same swelling of the head was noticed in the salamanders, but with them it is not so marked.

A single adult *Amblystoma punctatum*, that had been first anesthetized with chloroform, was placed in a solution equivalent to about 1 per cent. of formaldehyde, and was found to harden rapidly. There was, however, a very noticeable swelling of the whole body within twenty-four hours, while, at the same time, the costal furrows, as in the *Plethodon* specimens, became more marked. After about a week's immersion in the 1 per cent. solution, it was found that the bright orange-yellow spots of the live animal had very noticeably faded to light yellow. The specimen was then changed to a 4 per cent. solution and after an equal length of time the fading appeared to have gone no further, while the swelling was somewhat reduced. As it is the specimen is much better preserved than it would have been in alcohol.

Earthworms and the Arthropods were tried in all solutions. The former swell but slightly in the weaker solutions and contract very much less in the stronger ones than they would in alcohol, chromic acid or the other hardening agents. They harden in a $\frac{1}{4}$ per cent. solution as well as in the stronger ones or in pure formalin, the difference being one of time. In the Arthropods—sow-bugs and myriapods—the fluid in some of the experiments entered the body to such an extent as to stretch the animals out, leaving broad gaps between the harder parts of the segments. This stretching or swelling was first seen in the specimens in the 1 and 2 per cent. solutions, but sometime later those in all the solutions below 1 per cent. were fully as badly swollen, if not more so. Besides this the brownish colors of the animals became more faint, while the fluid became very much colored. This extraction of color is most noticeable with the $\frac{1}{4}$ per cent. solution.

Slugs placed in $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 4, 8, 10 and 20 per cent. solutions seem equally well preserved. In the 1 and 2 per cent. solutions the head is

a little more distended than in the others. When first placed in the solution they gave off considerable slime, but this became perfectly transparent so, as noted by Blum, the form and colors of the animals were not obscured.

With salamander blood some startling effects were obtained. A few drops of blood were placed on a slide in a 1 per cent. solution of formaldehyde and watched under the microscope. The corpuscles and especially the nuclei were seen to swell rapidly. The nuclei became as large almost as the original corpuscles and were seen to pop out of the corpuscle like a grape from its skin. The envelopes then became very pale and finally disappeared from view, the nuclei, however, remained very distinct. Staining with Erlich-Biondi mixture showed that the body of the corpuscles had simply been rendered very transparent by the solution, while immersion in alcohol coagulated the fibrin into an opaque, straw-yellow mass, and brought the corpuscles faintly back into view. This explains the phenomenon of the return of the color of blood vessels noted by Blum as due to the coagulation of the fibrin which may also be stained somewhat by the color drawn from the corpuscles. The same experiment was performed with a 4 per cent. formaldehyde solution in place of the weaker one and the swelling effect found to be very much lessened, none of the nuclei becoming as large as the corpuscle nor escaping, otherwise the results were the same.

After this an earthworm was anesthetized with chloroform, placed in a 1 per cent solution of formaldehyde for several hours and afterwards removed to a 2 per cent. solution. There it remained for an equal length of time, when it seemed perfectly hardened and was removed to Czoker's alum cochineal. On the following day pieces were rapidly dehydrated in 70 per cent. and 95 per cent. alcohol and imbedded in paraffin. Sections made from them showed all micro-anatomical details perfectly preserved. Nothing had stained but the nuclei which had all become very much swollen, giving the whole section a bright red-purple appearance. So decidedly had they swollen that in both series of the muscular system, and in the septa where they are not ordinarily visible, nuclei were shown very distinctly and in large numbers. Careful observation showed the nucleolus a little more deeply colored than the rest, while the chromatic filaments seemed swollen and less distinct.

Sections were also made of an earthworm hardened in pure formalin and no swelling whatever was to be noticed, while all cytological detail was remarkably well preserved.

To counteract the swelling effect of the weak solutions alcohol was employed. A 5 per cent. solution of formaldehyde in 50 per cent. alcohol hardened pieces of earthworm and cat liver very rapidly, so that on the day following their immersion, sections could be obtained by the paraffin method. Here the nuclei were found not to have swollen noticeably, if at all, while nuclear detail was plainly brought out by staining. In the pieces as a whole, there was neither swelling nor shrinkage, while the liver did not become as pale as it would have in alcohol.

For stains alum cochineal, Erlich-Biondi, Orth's picro-carminate of lithium, Erlich's acid hæmatoxylin, picric acid, fuchsin and saffranin were tried and their action found not to be very much if at all interferred with by the formaldehyde. In one instance a piece of an earthworm was placed in equal parts of 2 per cent. formaldehyde and alum cochineal. On the following day it had been little more than superficially reddened, while a piece that had been removed from the same solution (2 per cent.) of formaldehyde and left for the same length of time in undiluted alum cochineal had stained perfectly.

In *Nostoc* the dark yellowish-green has been extracted in 4 per cent formaldehyde leaving the filaments as seen with the naked eye of a whitish or very light green, while a dark green pond scum after immersion in the fluid for nearly two weeks has changed slightly to brownish-green. Still it is not unlike old specimens of the same and similar material that one often finds in ponds.

In conclusion it may be said that for general purposes, solutions of at least more than 2 per cent. must be used in order to avoid the swelling and decolorization of specimens, that from 4–8 per cent. will give the best results. For histological purposes formalin combined with alcohol will give better results than either used alone; while the weak (1–2 per cent.) solutions by swelling nuclei may serve the very important special purpose of demonstrating the presence of cells not otherwise readily distinguished.

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